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INTRODUCTION

The biological cell is the basic building block of life. Evolved from simple bacteria in the sea to the sophisticated humans, all organisms are built up from these carefully organized chemical factories. Despite the enormous variety of organisms, the chemical composition of the cell is remarkably conserved: the basic components DNA, proteins, sugars, and lipids can be found in each and every living cell.

An important structural component of the cell is the lipid bilayer, which constitutes a membrane that is impermeable to almost all molecules.¹ This nanometer thin lipid membrane acts as an effective barrier that compartmentalizes the cell into many types of organelles (see Fig. 1.1). Next to this, membranes provide a platform for proteins that drive biochemical processes, such as photosynthesis in chloroplasts, energy production in mitochondria, and signal transduction in the brain. Thus, lipid membranes fulfil a crucial structural roll in living cells.

This biological function is tightly related to the shape of the membrane, which varies strongly due to its flexible nature. For instance in the growth and migration of cells, or in more local processes such as vesicle budding (see Fig. 1.2a), membranes undergo dramatic shape changes.² This shape change is, among other things, guided by membrane deforming proteins.²⁻⁴ For instance, vesicle budding is initiated by membrane deforming proteins that create the initial dent in the membrane.^{5,6} Intriguingly, the opposite has also been observed: some proteins are sorted by the membrane, for example by spontaneously concentrating on the outside of membrane tubes.^{7,8} Thus, membrane-deforming proteins shape the membrane, and at the same time respond to the shape of the membrane.

A consequence of this two-way interaction is a force between membrane-deforming proteins that is mediated by the membrane. When one protein deforms the membrane, the other protein feels this deformation, leading to an effective attraction or repulsion between them. This membrane-mediated interaction has been predicted repeatedly, but has never been observed directly, mainly because proteins are too small to be resolved using conventional microscopy techniques. In this thesis, I address this open issue by employing micron-sized colloidal particles to experimentally measure forces between membrane deformations.

In Chapters 2 and 3, methods for extracting local forces from video images of colloidal particles are described. Then, in Chapter 4, the development of colloidal particles that strongly attach to specific lipid membranes is described. These are then used in Chapters 5 and 6, in which membrane mediated forces and assembly pathways between membrane attached colloidal particles are investigated and quantified. Finally, in Chapters 7 and 8, the preparation of micron-sized oil droplets is studied and their use as lipid monolayer support is demonstrated.

In the remaining sections of the Introduction, the necessary concepts and context for this thesis will be delineated. For a Dutch summary of the most important results and the main conclusions, see page 135.

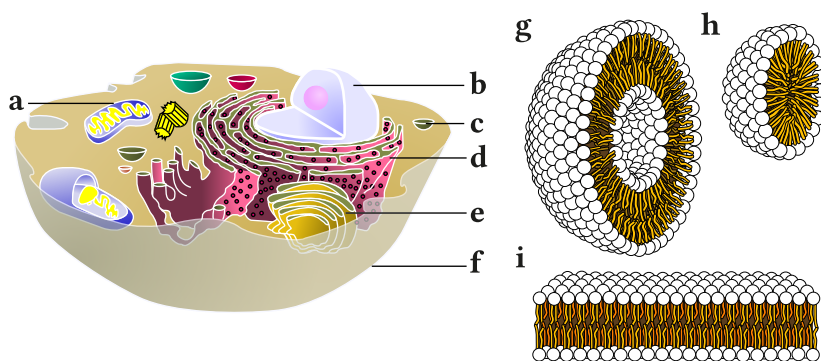


Figure 1.1. Cross-section of a eukaryotic cell (left) and self-assembled lipid structures (right). A selection of cellular organelles are denoted with letters, as follows: (a) mitochondria, (b) nucleus, (c) vesicle, (d) endoplasmic reticulum, (e) Golgi apparatus, and (f) the plasma membrane. In (g) to (i), lipids are denoted by a circle with two attached lines, which respectively denote the hydrophilic head, and the two hydrophobic fatty acid tails. (g) Lipids assemble into bilayers that close themselves into a vesicle. (h) Wedge-shaped lipids assemble into micelles. (i) Lipids with an aspect ratio near 1 assemble into planar bilayers. Images were reproduced from refs. [9, 10].

1.1 Lipid membranes: a biological view

Membrane structure Lipid molecules that form the lipid membrane are amphiphilic: their head group is hydrophilic, while their tail groups are hydrophobic.¹ Depending on their head-to-tail size ratio, they therefore self-assemble in water into structures with their head groups facing outwards. See Figure 1.1g–i. Cellular membranes mostly consist of phospholipids, which have a phosphate-based head group and two fatty acid tails. As lipids are not connected to one another, they behave like a fluid.¹¹ This makes most proteins that are associated with the membrane also mobile, which enables their biological function.

Membrane deforming proteins Cellular membranes are shaped by the interplay of different lipids and membrane proteins.² Many dynamic membrane processes are governed by proteins that attach to the membrane and induce local deformation. For example, the transport of nutrients and signalling molecules in and out of the cell requires the budding of vesicles from the plasma membrane. See Figure 1.2a–d. This important process involves the creation of negative and positive curvatures in the membrane, which is made possible by membrane-associated proteins.

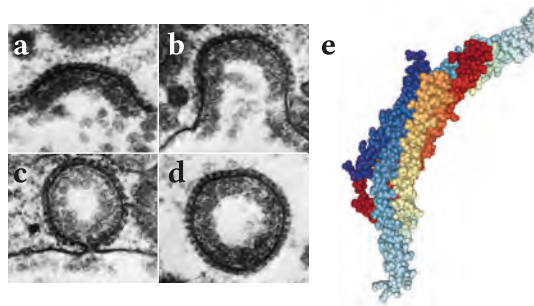


Figure 1.2. Membrane proteins shape the membrane. (a)–(d) Four different stages of vesicle invagination (endocytosis) in an immature chicken egg cell. In these electron micrographs, a cross-section of the membrane is visible with the cell inside on the top. The scale bar denotes 100 nm. Reprinted from ref. [12] with permission from The Company of Biologists. (e) Three-dimensional structure of another membrane-deforming protein: the Amphiphysin BAR domain. This structure was generated from the protein database¹³ with NGL Viewer.¹⁴

Perhaps the most pronounced example of membrane-deforming protein are the banana-shaped BAR domain containing proteins.^{13,15} See Figure 1.2b. These protein domains associate with lipid membranes and deform them locally due to their shape and specific binding characteristics. BAR domains are thought to play a role in the initiation of filopodia⁸ and in the stabilization of membrane tubes.¹⁶

In these examples, not one, but many proteins together result in membrane deformation. Computer simulations^{4,17–19} have predicted that through the membrane deformation of a single protein, other proteins are repositioned and membrane deformation increases, which eventually leads to a biological function such as vesicle budding. To understand this collective effect of local membrane deformations on the membrane shape, it is important to know how a pair of deformations interact with one another via the membrane. In Chapters 2 to 5, the development of an experimental model system that measures this membrane-mediated force is described.

Microparticles In this experimental model system, colloidal particles are used to deform the membrane and study the membrane-mediated interactions. These solid particles of micrometer size have been found to attach to lipid membranes, deform them, and aggregate on them, both on vesicles^{20,21} and on living cells^{22–24} (see Fig. 1.3). Due to

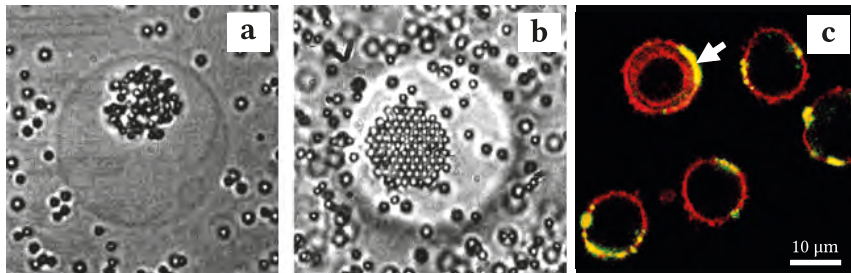


Figure 1.3. Aggregation of microparticles on lipid membranes. Bright field images of (a) disordered and (b) ordered aggregates of micron-sized latex particles adsorbed on bilayer vesicles. (c) Fluorescence images of polymeric particles (green) adsorbed on KG1a cells (red). The arrow indicates an aggregate of particles. (a) and (b) were reprinted from ref. [20] with permission from AAAS. (c) was reprinted from ref. [22] with permission from Elsevier.

their increased use in paints, cosmetics, and pharmacological applications, these microplastics are present more and more in the environment.²⁵ Therefore, studying the interaction between microparticles and lipid membranes is not only useful as model system for membrane-mediated forces, but also to address the question how microplastics influence lipid membranes. In Chapter 5 we will describe how microparticles attach to lipid membranes, and in Chapter 6, we will systematically investigate permanent assembly pathways that lead to aggregation of microparticles on lipid membranes.

1.2 Lipid membranes: a physical view

The physical model The lipid membrane can be seen as a two-dimensional fluid of lipid molecules with a certain surface viscosity and area compressibility modulus. In addition to that, membranes can curve in the third dimension. The compressibility modulus of both membrane monolayers gives bilayers a certain resistance against bending, which is expressed in the bending rigidity. This mix of liquid and elastic properties make lipid membranes unique physical objects that require a separate theoretical description. The generally accepted model for the energy of a symmetric lipid membrane was formulated by Canham and Helfrich in the 70s as follows:^{26,27}

$$U = \iint [\sigma + \kappa H^2 + \kappa_g K] dA, \quad (1.1)$$

with U the energy, σ the membrane tension, κ the bending rigidity, κ_g the Gaussian bending rigidity, H the mean curvature, and K the Gaussian curvature (see Fig. 1.4a). For

a closed surface like a vesicle (Fig. 1.1g), it follows from the Gauss-Bonnet theorem that the integral over the Gaussian curvature contributes a constant energy. As constant contributions to the energy are irrelevant, this term is typically left out.

Membrane tension Surface tension is defined as the free energy increase associated with the increase of surface area. For example in a water/air interface surface tension is always positive so that the surface tends to contract, minimizing its surface area. This process is driven by the transport of water molecules from the interface to the bulk. For lipid membranes, however, we do not have a bulk and therefore, transport of lipid molecules is only possible if there is a reservoir of lipids at the membrane edges. Lipid vesicles in general have no such reservoir and therefore they have a constant number of surface molecules, which is a situation that is uncommon for conventional interfaces.

Elastic extension then becomes the only mechanism through which the membrane surface area can change. This typically requires high tensions, which can be achieved through high osmotic pressure differences or by suction through a micropipette,²⁸ until the vesicle bursts. Outside of this stretched regime, vesicles essentially have a constant area, so that the Helfrich energy (Eq. 1.1) of a vesicle simplifies to:

$$U_{vesicle} = \iint \kappa H^2 dA. \quad (1.2)$$

Nevertheless, in literature there is often a membrane tension assigned to deflated vesicles. While the surface area of a vesicle cannot change, the projected surface area can, and through thermal fluctuations, this can be expressed in an effective membrane tension which is related directly to the (real) area-to-volume ratio of the vesicle.²⁹ This entropic surface tension is used throughout this thesis as the membrane tension.

Membrane-mediated forces A single membrane-deforming object introduces a deformation field in the membrane.³⁰ See for example Fig. 1.4b–c for two membrane-deforming objects. When two of these objects approach each other, the induced membrane deformation fields interact resulting in effective attractive or repulsive forces between the deformations. This membrane-mediated force stems from the minimization of the membrane free energy.

Since the 90s, there have been many predictions of membrane-mediated forces, including forces mediated by membrane thickness modulation,³¹ Casimir-type fluctuation mediated forces,³² and interactions driven by the phase separation of multicomponent membranes.³³ The main focus of previous work however involves a membrane-bending mediated force, that follows from the minimization of the Helfrich energy (Eq. 1.2). By linearising this equation, which is valid only for small membrane deformations, repulsive interaction forces have been predicted.¹⁷ For large deformations, this approach is however not valid and an effective field theory approach can be taken to describe the force between two membrane inclusions. However, this approach is not able to predict even the sign of the membrane-mediated force.³⁴

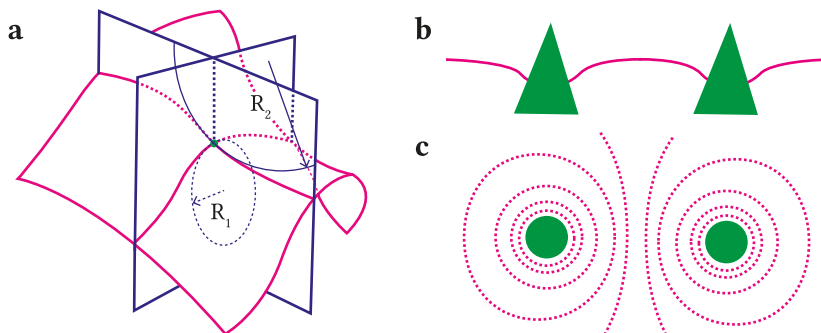


Figure 1.4. Illustrations of membrane deformations. (a) Sketch of an arbitrarily shaped membrane patch (magenta) with the radii of curvature in a point (green) drawn on planes perpendicular to the surface. The mean curvature H is defined as the average of $1/R_1$ and $1/R_2$ and the Gaussian curvature K as the product between $1/R_1$ and $1/R_2$. Curvature also has a sign. If the vesicle inside is below the sketch, R_1 is positive and R_2 is negative. (b) Cross section of a membrane with two inclusions (green) that induce local curvature (c) Top view with the contours of equal height as dashed lines, showing that the deformation fields interact with each other. These illustrations are sketches and not numerical results.

Computer simulations have extended the theoretical descriptions and have been able to quantitatively predict membrane bending mediated forces. The predicted forces between membrane-embedded objects can be repulsive or attractive, depending on the extent of the deformation and the distance between the objects.³⁵ Simulations involving many membrane-deforming objects have shown that these interactions can indeed lead to global deformation of the membrane, such as line formation, tubulation, and vesiculation.^{4,18,19,36} In Chapter 5, this membrane-mediated force is quantified experimentally and compared to these numerical predictions.

1.3 Lipids in self-assembly

Next to their presence in biological processes and in food science, lipids have also found their way into a variety of technological applications, such as targeted drug delivery,³⁷ surface passivation in microfluidics,³⁸ nanoporous materials, electronics, and photonics.³⁹ In this thesis we will focus on yet another application of lipids: the production of self-assembled colloidal materials.

Colloidal self-assembly is a promising tool for creating new materials from the bottom up. To engineer a self-assembled colloidal structure, building blocks with specific and directional bonding sites are required.⁴⁰ Important steps have been taken to create these controlled building blocks with specific linkers,⁴¹ however the self-assembly of complex materials remains a challenge because of kinetic arrest that occurs when

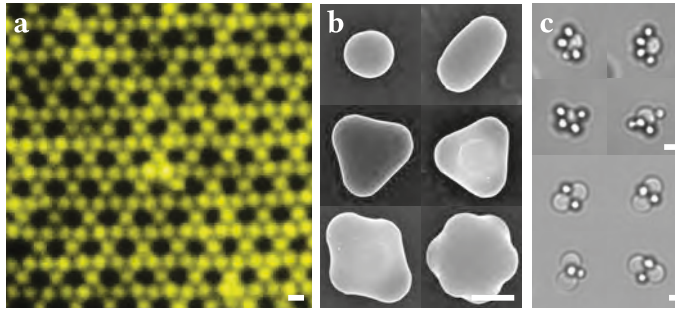


Figure 1.5. Examples of self-assembled colloidal structures. (a) Self-assembled colloidal Kagome lattice, which has useful optical properties. (b) Colloidal clusters with controlled shape, self-assembled from 1 to 6 spheres.⁴³ (c) Two examples of flexible self-assembled structures.⁴⁴ On the top, four snapshots of a colloidal ball joint is shown, and on the bottom a hinge joint. Scale bars denote 1 μm . (a) was adapted from ref. [45] with permission from Macmillan Publishers, copyright 2011.

building blocks bind together, yielding open random packings.⁴²

To controllably assemble structures such as displayed in Figure 1.5, building blocks need to be able to rearrange after connecting.^{46,47} One way to achieve this bond flexibility is the use of lipid-coated emulsions instead of solid particles as building blocks.^{48,49} Here, the lipids provide the specific linking capability, while they are mobile laterally on the emulsion surface. Oil-in-water emulsions however typically have a wide size distribution, and are therefore less useful to build regular structures.

In this thesis, lipid-coated 3-(trimethoxysilyl)propyl methacrylate (TPM) microdroplets were investigated as a possible material to produce dynamic colloidal assemblies. TPM has the advantage that it spontaneously forms emulsions in water with a narrow size distribution.^{50,51} In Chapter 7 we will investigate this spontaneous emulsification to be able to control the resulting droplet size. Then, in Chapter 8, we will study the mobility of lipids, DNA-linkers, and colloidal particles on the surface of these TPM emulsion droplets.

